

(a) exposing a biological sample to a nucleic acid primer capable of hybridizing with a nucleic acid, said primer having a covalently-attached donor molecule comprising a fluorophore or a fluorescent dye;

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(b) performing a primer extension reaction in the presence of a dideoxy nucleotide complementary to the target nucleotide, said dideoxy nucleotide having a covalently-attached acceptor molecule comprising a fluorophore or a fluorescent dye, said acceptor molecule being capable of being activated through fluorescent energy transfer from said donor molecule so as to produce a detectable fluorescent signal when said dideoxy nucleotide is incorporated into a product resulting from the primer extension reaction;

(c) determining the presence of said fluorescent signal, said presence being indicative of incorporation of said dideoxy nucleotide into the primer extension product; and

(d) determining the presence of said target nucleotide as indicated by the incorporation of said dideoxy nucleotide into the primer extension product.

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4. (Amended) The method of claim 1, wherein said extension reaction is performed in the presence of at least two different dideoxy nucleotides, each comprising a different acceptor molecule that produces a distinct fluorescent signal upon activation.

a3
10. (Amended) The method of claim 1, wherein said fluorescent dye is selected from the group consisting of 6-carboxyfluorescein (FAM), 6-carboxy-X-rhodamine (REG), N₁, N₁N¹-tetramethyl-6-carboxyrhodamine (TAMARA), 6-carboxy-X-rhodamine (ROX), fluorescein, Cy5® or LightCycler-Red 640.

11. (Amended) The method of claim 1 wherein said donor molecule comprises 6-carboxyfluorescein (FAM).

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12. (Amended) The method of claim 11 wherein said acceptor molecule comprises, 6-carboxy-X-rhodamine (ROX).

a4

15. (Amended) The method of claim 1 wherein said dideoxy nucleotide is a 2'3'-dideoxy nucleotide triphosphate selected from the group consisting of ddATP, ddCTP, ddGTP, ddTTP and ddUTP.

a5

18. (Amended) The method of claim 1, wherein said target nucleotide is present as a result of a nucleic acid mutation.

a6

20. (Amended) The method of claim 4, wherein said target nucleotide is present at a single nucleotide polymorphic locus.

a7

24. (New) The method of claim 1, wherein said target nucleotide is absent as a result of a nucleic acid mutation.

Basis for the Amendments

Claims 2, 3, 5-9, 13, 14 and 21 have been canceled without prejudice and without any intention to abandon the subject matter claimed in any one or more of those claims. Indeed, Applicants may pursue claims drawn to that subject matter in a continuation application.

Claims 1 and 20 have been amended to claim an embodiment of a method in accordance with the instant invention in which the presence of a target nucleotide on a nucleic acid is determined, and to specifically recite a donor molecule that is covalently-attached to a primer, and an acceptor molecule that is covalently-attached to a dideoxy nucleotide. In accordance with the claims, as amended, the acceptor molecule is capable of being activated through fluorescent energy transfer from the donor molecule if the dideoxy nucleotide is incorporated into an extension product of the primer. The amendments are supported throughout the specification as filed, as well as in the canceled claims 2, 3, 5-9, 13, 14 and 21.